POTASSIUM AND SODIUM REGULATION IN AN INTERTIDAL CRAB

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This paper will show that when the crab *Pachygrapsus crassipes* is exposed to a simple osmotic stress it regulates its blood sodium and potassium equally well. However, more potassium than sodium is exchanged between animal and external medium for a given alteration in the blood, which means that a source of potassium other than the blood is contributing to the exchanges. Also, evidence will be produced indicating that sources other than the blood contribute to sodium exchanges between crab and medium, thus suggesting the presence of adaptive salt pools, a phenomenon postulated by Hukuda (1932).

Much work has been done, especially on mammals, demonstrating that the ionic concentration in tissues can be altered by experimentally varying the concentration of ions in the environmental fluid. Such studies are reviewed by Manery (1954) and Harris (1956). Krogh (1939), Prosser *et al.* (1950) and Beadle (1957) consider the subject of ionic and osmotic regulation in aquatic animals, but these reviews are concerned chiefly with changes in the blood which are effected by alterations in the concentration of the external medium. Little information is available concerning the fate of ions entering an aquatic animal from a hypertonic external medium, nor the source of ions leaving an animal to a hypotonic medium. Prosser *et al.* (1955) have demonstrated the final sodium and potassium blood concentration of the crab *Pachygrapsus crassipes* after it is exposed to diluted or concentrated sea water. However, the total exchange of these two ions between animal and medium has not been shown. Thus, it could not be stated, for example, whether the loss of blood ions by an animal to a hypotonic medium represented the total loss by the animal or whether sources other than the blood were contributing to the loss.

Materials and Methods

The shore crab, *Pachygrapsus crassipes* Randall, used in this investigation was collected at Newport and Laguna, California. *Pachygrapsus* is particularly suitable for this type of study because it can regulate osmotically in dilute as well as concentrated sea water. Also, it can live out of water for extended periods (Jones, 1941; Prosser *et al.*, 1955; and Gross, 1955). All specimens used were between molts and were mature, none weighing less than 20 grams.

Sodium and potassium concentrations were measured by means of a Beckman flame photometer using a standard which contained potassium and sodium approximating the respective proportions in the blood. Samples of blood of approximately 0.05 ml. were extracted serially from individual crabs, measured, in calibrated capillary tubes and diluted in 25 ml. of distilled water. Such samples were used

directly in the flame photometer. Known quantities of sodium added to the blood thus treated could be recovered within 2% with concentrations of about 500 mEq./l. and potassium could be recovered within 10% of concentrations of around 10 mEq./l.

The exchange of ions between animal and external medium was determined as follows: Blood from a crab recently removed from sea water of known concentration was analyzed for sodium and potassium. The same specimen was kept out of water for a brief time to permit the rapid coagulation of blood and then was immersed in a known volume of a different salinity having been rinsed first in that new salinity. The volume of medium varied from 50 ml. to 100 ml., depending on the size of the specimen. All animals could raise themselves out of the water and thus usually were not immersed completely. After a period of immersion (24–48 hours) during which time adequate precautions were taken against evaporation, the crab was removed, and its blood as well as external medium were analyzed again for potassium and sodium. Thus determinations of sodium and potassium were made on crabs both before and after exposure to the experimental media. The following experimental treatments were studied:

a) Transferred from normal sea water to dilute sea water (25% or 50%).
b) Transferred from normal sea water to concentrated sea water (approximately 150%).
c) Acclimated for 1–2 days to 50% sea water, then transferred to approximately 150% sea water.
d) Acclimated for 1–2 days to approximately 150% sea water, then transferred to 50% sea water.

The effects of desiccation on the blood concentrations of sodium and potassium also were investigated. Blood from crabs freshly removed from normal sea water was analyzed for potassium and sodium; the crab was then blotted dry, weighed and placed in a chamber at 15° C. for desiccation. After a period ranging from 24 to 72 hours, the animal was dipped in sea water to replace the evaporated branchial fluid, blotted and weighed. The blood then was analyzed again for sodium and potassium. Blood from a few partially desiccated crabs was analyzed for potassium and sodium; then the animal was desiccated further, and its weight change between the two desiccated conditions was measured without dipping as above, since there was little or no water remaining in the branchial chamber after partial desiccation. Then the blood was analyzed again for sodium and potassium. Since the effects of desiccation on the ionic concentration of the blood per unit weight loss by evaporation were not significantly different for the two above methods, it can be concluded that branchial fluid is accurately replaced by dipping and the weight losses caused by evaporation do not include the branchial fluid. Thus the blood sodium or potassium concentration change could be determined for a given weight loss caused by evaporation.

All of the above treatments were endured by most of the crabs tested which seemed to recover when returned to normal sea water. The repeated blood sampling resulting in a total loss of not more than 0.2 ml. does not seem to impose too great a stress since a 30-gram crab containing about 5 to 10 grams of blood can survive the loss of 1 ml.

Results

Table I, which presents the blood potassium and sodium concentrations of normal *Pachygrapsus,* freshly removed from the sea, shows the mean potassium concentration as 7.43 mEq./l. and the mean sodium concentration as 483.3 mEq./l. While the latter value is in close agreement with Prosser *et al.* (1955), the value for potassium is considerably less than 12.1 mEq./l. reported by the above workers.

Table I

Blood concentrations of crabs after treatment

Treatment		No. of crabs	Final blood concentration		Blood changes		
			Mean mEq./l.	S.D.	Mean mEq./l.	S.D.	% original
100% sea water to 25%* or 50% sea water	Na	28	373	37.0	−107	38.0	−22.8
	K	22	6.03	2.16	−2.25	2.11	−28.1
100% sea water to 150% sea water	Na	25	574	26.1	+99.2	26.1	+20.7
	K	24	9.71	1.54	+2.67	1.25	+38.3
50% sea water to 150% sea water	Na	12	572	37.2	+180	37.1	+43.5
	K	12	9.21	1.19	+3.26	0.90	+58.2
150% sea water to 50% sea water	Na	14	406	37.3	−174	61.2	−29.7
	K	14	6.78	1.35	−2.92	1.71	−29.2
		No. of crabs	Means			S.D.	
Normal crabs freshly removed from the sea	Na	36	mEq./l.		483.3	17.3	
			% sea water		103		
	K	36	mEq./l.		7.43	0.72	
			% sea water		74		

* Blood concentrations of crabs immersed in 25% sea water were not significantly different from those immersed in 50% sea water. This is because the former were immersed for briefer periods.

This difference is even more significant because the potassium values obtained in the present investigation are less than those in the external medium (9.8 mEq./l.) whereas the values of Prosser *et al.* were more than the medium. The latter studies were made at Pacific Grove, California, about 300 miles north of the Laguna area where specimens for the present investigation were collected. Possibly temperature is the significant difference.

Not only is the blood potassium of *Pachygrapsus* less concentrated than the potassium of normal sea water, the natural medium of this crab, but it remains less concentrated even when immersed in media as dilute as 50% sea water, *i.e.*, there is a tendency for the blood potassium to remain less concentrated than that in the medium. For 28 crabs immersed 24–48 hours in 50% sea water (salts were lost from the animal, increasing the medium concentration) the mean ratio, blood potassium (mEq./l.)/medium potassium (mEq./l.) was 0.897, S.D = 0.19 which is significantly less than one, $P < 0.01$. As expected, the blood potassium is likewise less concentrated in hypertonic media.

Blood sodium on the other hand, which contributes about half of the blood osmotic pressure, was maintained above the sodium concentration of dilute media and below sodium concentrations in concentrated media, indicating active regulation of this ion as described by Prosser *et al.* (1955). Thus under the conditions of these experiments neither blood sodium nor potassium achieves concentrations equal to those of the external medium. Neither does the blood become isotonic to the external medium (Jones, 1941; Prosser *et al.*, 1955; Gross, 1957).

Table I also shows the ionic alterations that occur under various treatments in aqueous media. These values are presented to demonstrate the magnitude of blood ion changes, but they should not be considered comparable to those values reported in other investigations where animals were immersed completely in large volumes of water. The prime objective of the present investigation is to demonstrate the ionic change that occurs in the medium per given ionic change in the blood of the animal. It should be pointed out that the dilution and concentration of the blood of *Pachygrapsus* such as is shown in Table I is effected by salt exchange, not water (Gross, 1957). This must mean that a loss of ions in the external medium is essentially the same as an injection of salts into the animal. On the other hand a gain of ions in the external medium is the same as a removal of ions from the animal. We cannot say at this point whether or not those exchanges occur only between blood and external medium.

Now the "apparent volume of distribution" (Winkler *et al.*, 1943) in the animal for each ion can be estimated from the following equation,

$$V = M/P \times 100,$$

where: V = "apparent volume of distribution" in % body weight;

$$M = \frac{\text{weight of medium}}{\text{weight of animal}};$$

$$P = \frac{\text{change in blood ion concentration (mEq./l.)}}{\text{change in medium ion concentration (mEq./l.)}}$$

(the observed ratios, P are presented in column 2, Table V, corrected to an M value of 1.0).

Table II shows the effect of desiccation on the blood of crabs. After a crab is desiccated, the "apparent volume of distribution" can be estimated by the equation,

$$V = \frac{E}{C_1/C_2\text{-}1},$$

TABLE II

Sodium and potassium increases in the blood caused by desiccation

	No. of crabs	Change in ion concentration (% original concentration) per 1% body weight loss*		
		Mean	S.D.	95% fiducial limits
Na	84	+2.20	0.71	2.05– 2.35
K	50	+8.68	11.75	5.36–12.00

* By evaporation.

where E = % weight change caused by evaporation,

C_1 = initial blood ion concentration (mEq./l.),

C_2 = final blood ion concentration (mEq./l.).

Table III gives the "apparent volume of distribution" for sodium for the various treatments. Here it can be seen that the volume in question varies with the treatment. Thus by the desiccation method it averages 48.9% body weight. When crabs were transferred from normal sea water to dilute sea water the volume av-

TABLE III

"Apparent volume of distribution" for sodium

	Treatment	No. of crabs	Volume in % body weight	S.D.
A	Desiccation	84	48.9	12.8
B	Normal sea water to Dilute sea water	28	39.0	12.8
C	Normal sea water to Concentrated sea water	25	37.9	11.7
B + C	Normal sea water to Dilute or concentrated sea water	53	38.5	12.1
D	150% sea water to 50% sea water	14	46.9	4.52
E	50% sea water to 150% sea water	12	44.8	8.56
D + E	50% or 150% sea water to 150% or 50% sea water	26	45.9	6.55

erages 39.0%; where animals were transferred from normal sea water to concentrated sea water, 37.9% body weight. The latter two means are not significantly different from each other, so the values for the two treatments were combined, and these averaged 38.5% body weight. This value was shown by "t" evaluation to be significantly different from the above 48.9% value estimated by the desiccation method, $P = 0.003$. When large blood changes were effected by first acclimating the animal to either dilute or concentrated sea water (50 or 150%), then transferring it to the opposite stress and measuring the resultant changes in the blood

TABLE IV

Relative sodium and potassium concentration changes in the blood caused by osmotic stress

	Treatment	Blood sodium change (% original) / Blood potassium change (% original)		
		No. of crabs	Mean of ratios	S.D.
A	100% sea water to 25% or 50% sea water	22	1.24	1.31
B	100% sea water to 150% sea water	23	0.72	0.57
A + B	100% sea water to dilute or concentrated sea water	45	0.97	0.91
C	50% sea water to 150% sea water	12	0.83	0.31
D	150% sea water to 50% sea water	14	0.92	0.22
C + D	50% or 150% sea water to 150% or 50% sea water	26	0.88	0.26

and external medium of the animal, the "apparent volume of distribution" averaged 45.9% body weight. There was no significant difference between values calculated on crabs started in 50% sea water and those initially placed in 150% sea water. However, the volume 45.9% body weight was shown to be significantly different from 38.5% body weight determined for crabs transferred from normal sea water to various stresses (B + C), $P = 0.0004$. Yet, the "apparent volume of distribution" determined by (A), the desiccation method (48.9% body weight), was not significantly different from 45.9% body weight.

Table III reveals also that the variance in values for "apparent volume of distribution" is markedly reduced when the crab is exposed to extreme osmotic stresses

(*e.g.*, transferring from 50% to 150% sea water). This merely means that a large sodium change effected by the extreme stress can be measured with greater precision than a small sodium change. That is, the percentage error would be larger for the determination of a small change than for a large change since the accuracy of the method is constant. Thus the large variances observed for the desiccation method and for the moderate stress (B and C, Table III) method are believed to be the result of experimental error and not physiological variation.

The "apparent volume of distribution" for potassium in most cases was calculated to be greater than 100% body weight by the immersion method and averaged about 13% body weight by the desiccation method. It thus becomes clear that the "apparent volume of distribution" has little morphological significance even when referring to a specific ion. The following sections will show that differences in values for "apparent volume of distribution" are indications that sources of ions within the crab other than the blood are participating in exchanges with the medium.

First, in Table IV it can be seen that under various aqueous osmotic stresses the blood potassium changes of the crab are approximately equal to the blood sodium changes, percentage-wise. Of the four treatments represented in Table IV only in the case where crabs are transferred from normal sea water to concentrated sea water is the ratio, sodium change (% original)/potassium change (% original) significantly less than 1.0, $P = 0.05$. The other values are not significantly different from 1.0, which means that in general, potassium and sodium are regulated approximately equally in the blood of *Pachygrapsus* when the crab is subjected to osmotic stress. It should be observed that values in Table IV are means of individual ratios, blood sodium change (% original)/blood potassium change (% original), not ratios of the mean blood changes presented in Table I. Any discrepancy between the mean of ratios and the ratio of means can be explained by the observed variances.

Now let us assume that the mean "apparent volume of distribution" for sodium in crabs exposed to moderate stresses, 38.5% body weight, represents a constant volume of fluid in the animal in which both sodium and potassium concentrations are equal to those of the blood. This particular hypothetical volume, which hereafter shall be referred to as "A–D volume," was chosen because it was determined under conditions of moderate stress and would be expected to be closer to a possible morphological space than a value obtained under conditions of extreme stress. That is, a moderate stress is closer to a normal condition than is an extreme stress.

If then a change in the quantity of an ion in the external medium of an animal were known, by knowing the concentration change in the blood and the volume, 38.5% body weight, it could be determined what fraction of an ionic exchange between crab and external medium appears in the "A–D volume." Table V, column 1 reveals that in the case of potassium less than half of a loss or gain by the medium (42% maximum) is calculated to be accounted for in the "A–D volume." This means that a tissue potassium pool (probably the intra-cellular space) is participating in the exchanges. Also in the case where the crab is exposed to extreme osmotic stresses, only part of the sodium change in the medium (84%) appears in the "A–D volume," again an indication of a salt pool. It has already been shown that the sodium exchanges which occur under extreme stress are significantly different from those occurring under moderate stress (see discussion of data

in Table III). Now with respect to potassium there is no such trend with increased stress. However, the calculated ratio, change in "A–D volume"/change in medium with respect to potassium for crabs transferred from normal sea water to dilute sea water (0.22) is significantly less than 0.38, calculated for animals

TABLE V

Sodium and potassium exchanges between Pachgrapsus and ambient stress media

	Treatment		No. of crabs	(1) Calculated Change in "A-D volume" (mg.) / Change in medium (mg.)	(2) Observed Change in blood (mEq./l.) / Change in medium (mEq./l.)*	
				Mean	Mean	S.D.
A	100% sea water to 25% or 50% sea water	Na	28	0.98	2.56	0.82
		K	20	0.22	0.56	0.43
B	100% sea water to 150% sea water	Na	25	1.10	2.63	0.80
		K	24	0.38	0.998	0.70
A + B	100% sea water to dilute or concentrated sea water	Na	53	1.00**	2.60**	0.82
		K	44	0.31	0.80	0.62
C	50% sea water to 150% sea water	Na	12	0.86	2.23	0.43
		K	12	0.33	0.87	0.50
D	150% sea water to 50% sea water	Na	14	0.82	2.13	0.22
		K	14	0.42	1.08	0.35
C + D	50% or 150% sea water to 150% or 50% sea water	Na	26	0.84	2.18	0.32
		K	26	0.38	0.98	0.44

* Change in medium was caused by ions lost or gained by the crab. This change in all cases was corrected for a volume of medium equal to the weight of the crab because the ratio, weight of medium/weight of animal was not equal for all crabs.

$$** \frac{\text{"A-D volume"}}{\text{Medium volume}} \times \frac{\text{Change in blood (mEq./l.)}}{\text{Change in medium (mEq./l.)}} = \frac{\text{Change in "A-D volume" (mg.)}}{\text{Change in medium (mg.)}}$$

$$= 0.385 \times 2.60 = 1.00.$$

transferred from normal sea water to 150% sea water, $P = 0.01$, and also significantly less than 0.42, calculated for crabs treated by transferring from 150% sea water to 50% sea water, $P = 0.001$. On the other hand, 0.22 is not significantly different from the calculated value, 0.33, obtained for crabs treated by transferring from 50% sea water to 150% sea water. I have no explanation for this curious

fact that the ratio, change in "A–D volume"/change in medium for animals transferred from 100% sea water to dilute conditions, was lower than for some of the other treatments. The important conclusions that can be made from the data contained in Table V are: a) For a given change of the respective ions in the external medium more than twice as much sodium as potassium can be accounted for in the "A–D volume," which means that a potassium source other than the blood is participating in exchanges with the medium. b) The percentage of a sodium change in the medium which can be accounted for in the "A–D volume" decreases with increased osmotic stress, again suggesting the participation of a sodium pool. c) The salt pools quantitatively have the same role in hypertonic media as in hypotonic media.

Discussion

The validity of the above conclusions concerning the presence of salt pools does not depend on the validity of the value 38.5% body weight for "A–D volume." Qualitatively, the same conclusions can be reached from the data in column 2, Table V which are the *observed* ratios, change in blood (mEq./l.)/change in medium (mEq./l.) from which the *calculated* ratios, change in "A–D volume" (mg.)/change in medium (mg.) can be derived (column 1, Table V).

The different values for "apparent volume of distribution" (Table III) cannot be interpreted as a varying morphological space filled with fluid in which sodium is dissolved in concentrations equal to those of the blood. It has been established already (Gross, 1957) that *Pachygrapsus* does not gain or lose water significantly when immersed in an osmotic stress. Therefore, a sodium pool must be contributing to the exchanges which occur between animal and medium. It will be remembered that the values for "apparent volume of distribution" for potassium are usually more than 100% body weight. This suggests, of course, that the potassium of the intra-cellular fluid, known to be in relatively high concentrations, is participating in the exchanges with the medium, in effect acting as a potassium pool.

Now by assuming a constant volume of fluid in the crab ("A–D volume") in which sodium and potassium are dissolved in concentrations equal to those of the blood, we can arrive at a quantitative estimation of the role of the salt pool for a given ion exchange between animal and medium (Table V, column 1). Figure 1 illustrates further how a salt pool might function under conditions of *extreme* stress. Here a 100-gram crab whose "A–D volume" is 38.5% body weight is immersed in 100 ml of 50% or 150% sea water. Under conditions of presumed equilibrium this results in an assumed 20% alteration in the blood sodium; *i.e.,* from 500 mEq./l. to 400 mEq./l. when in the dilute medium, and from 500 mEq./l. to 600 mEq./l. in the concentrated medium. Since blood potassium is regulated approximately equally to blood sodium percentage-wise (Table IV), the blood potassium under the above conditions will also be altered 20%; *i.e.,* from 8.0 mEq./l. to 6.4 mEq./l. in the dilute medium and 8.0 mEq./l. to 9.6 mEq./l. in the concentrated medium.

Using the volume 38.5% body weight for "A–D volume," the above concentration changes can be converted to quantities of the two ions in milligrams. Also, knowing the volume of the external medium and the concentration change there (the ratios, blood change (mEq./l.)/medium change (mEq./l.) are presented in Table V, column 2) the ion loss or gains in the medium can be expressed in milli-

grams. Thus it can be seen in the diagram (Fig. 1) that while 106 mg. of sodium enter or leave the "A–D volume" (solid-lined arrow), a net change of only 89 mg. occurs in the "A–D volume," that is, 84% of the flux (see Table V, column 1, C + D). The remaining 17 mg. of sodium are fixed in the salt pool in the hypertonic medium or released from the salt pool in the hypotonic medium (dotted arrows), thus significantly contributing to the mechanism of maintaining ionic and osmotic homeostasis in the body fluids.

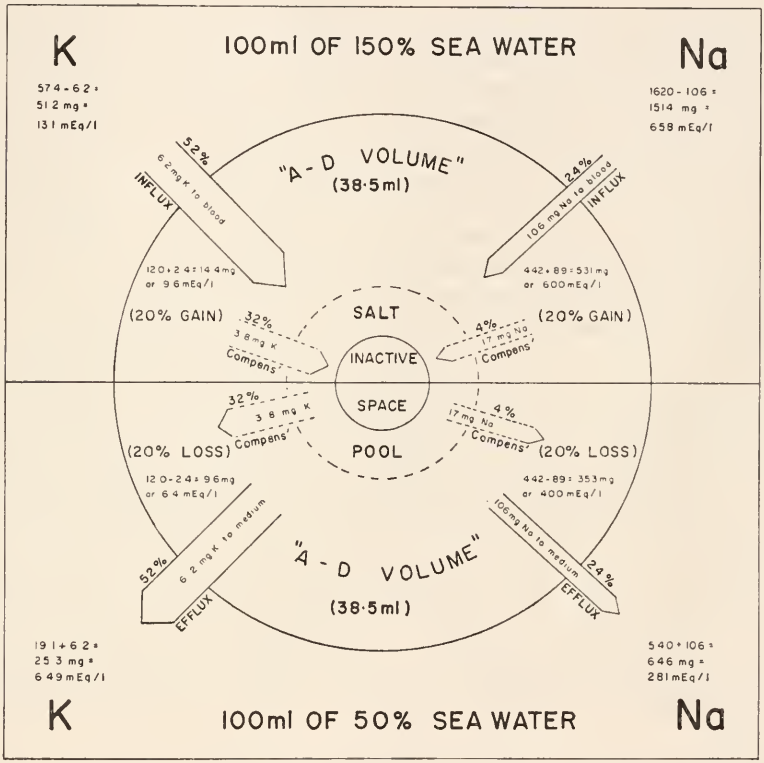

FIGURE 1. Suggested functional salt pool in a 100-gram crab immersed in osmotic stresses. Animal is represented by large circle; large square the external medium. Upper half of diagram illustrates net ion movements in 150% sea water; lower half; net ion movement in 50% sea water. Solid-line arrows indicate ion exchanges between external medium and "A–D volume" (assumed to be 38.5% body weight). Dotted arrows indicate ion exchanges between salt pool and "A–D volume." All percentages in diagram are with respect to initial blood concentrations (500mEq./l. for sodium; 8.0 mEq./l. for potassium). Numerical data are subject to small errors in rounding off. Compens' = compensation. The diagram may be read as follows: For example, for potassium loss in 50% sea water: a 100-gram crab assumed to have an "A–D volume" of 38.5% (explained in text) is found to lose 6.2 mg. of potassium to the medium, but its "A–D volume" potassium only goes down from 12.0 to 9.6 mg., a decrease of 2.4; the extra potassium is assumed to come from a pool and must be 6.2–2.4 = 3.8 mg. Therefore, the crab loses potassium equal to 52% of its initial "A–D volume" potassium, but the blood only decreases 20% and a compensation is calculated amounting to 32% of the initial potassium entering from the pool.

With respect to potassium, 6.2 mg. enter or leave the "A–D volume," but only a final change of 2.4 mg. remains in the "A–D volume," or about 38% of the flux (Table V, column 1). Again the remainder is fixed in or released from the salt pool.

It is possible that instead of compensatory exchanges occurring between salt pool and blood, salt fluxes occur directly between pool and external medium without passing into the "A–D volume." Such a phenomenon could yield the same results as presented in Table V, but it would seem negatively adaptive and also improbable because of the problem of transporting ions directly between pool and external medium through an exoskeleton which is relatively impermeable (Gross, 1957). It should be emphasized that such ion fluxes to and from the salt pool would be the same per unit blood change in 50% sea water as in 150% sea water. Passive transport from a pool containing the concentration of sodium and potassium permitting such fluxes would be extremely slow, especially through the exoskeleton. In the case of potassium, a greater net exchange occurs from the salt pool than from the "A–D volume" and presumably the blood which is separated from the external medium by tissues known to be permeable, *e.g.*, gills. While some flux of salts may occur directly between pool and medium, it seems more likely that the blood is traversed by the majority of the exchanged ions.

The events described above in Figure 1 have assumed conditions of extreme stress such as might occur by transferring an animal acclimated to 50% sea water into 150% sea water. The calculated role of the sodium pool in a lesser stress might decrease or become zero for the conditions in Figure 1 which were set up to explain the variation that occurs between two magnitudes of stress for the ratio, blood sodium change (mEq./l.)/medium sodium change (mEq./l.) (Table V, column 2). The morphological significance of "A–D volume" is obscure, but it seems possible that were the ratios in Table V, column 2, obtained under conditions of minute stress, the "apparent volume of distribution" for sodium would be smaller than 38.5% body weight and this smaller volume would have been chosen as the hypothetical constant "A–D volume." The principle would remain the same, however, namely that increased values for "apparent volume of distribution," for sodium with increased stresses does not indicate an increase in a volume of fluid, but rather participation of a sodium pool in the sodium exchanges between animal and medium.

The close agreement of values for sodium "apparent volume of distribution" obtained by the desiccation and the immersion method when animals were transferred from 50% sea water to 150% sea water or vice versa (extreme stress), is interpreted as a coincidence of values with possibly two different phenomena involved. Again, assuming the constant "A–D volume," 38.5% body weight, both the values, 45.9% body weight obtained from the extreme stress method and 48.9% body weight obtained from the desiccation method (Table III) can be explained as greater participation of sodium reservoirs. However, the value 48.9% body weight obtained by desiccation could also be explained by the participation of water pools which are inactive until conditions of desiccation exist when they are capable of replenishing water lost by evaporation. In either case, salt or water pool, the end result would be values for "apparent volume of distribution" which would be greater than those obtained under moderate immersion stresses (38.5% body

weight). In both cases there would be a tendency to maintain a constancy of blood which therefore would be an adaptive end result.

The large changes in the blood potassium relative to sodium that occur during desiccation (Table II) indicate that potassium passes from some sort of intrinsic supply into the blood. The inability of *Pachygrapsus* to regulate its blood potassium under conditions of desiccation may be an important factor limiting the terrestrial behavior of this crab. Gross (1955) discusses other limiting factors with respect to land habits of *Pachygrapsus.*

The nature of the above described salt pool may be merely the formed tissues responding to an osmotic or ionic stress, thus exchanging ions from the cytoplasm or cell surface when a gradient threshold is surpassed. This interpretation possibly is corroborated qualitatively by Shaw (1955) who demonstrated that muscle fibers of the crab *Carcinus* release both sodium and potassium when the animal is immersed in dilute sea water. Also, much information is available especially concerning mammals, demonstrating that the ionic concentrations of tissues can be altered by varying the concentration of ions in the environmental fluid. For example, muscle sodium will increase in an animal perfused with hypertonic sodium chloride solution; the liver shows gains in potassium when the plasma potassium is elevated, or muscle potassium will decrease if an animal is perfused with glucose solution. Such studies and similar studies concerned with isolated tissues are reviewed by Manery (1954) and Harris (1956).

It may well be that the findings of the present investigation are manifestations of the same general cellular mechanism, illustrated by the above mentioned perfusion experiments, *i.e.*, incidental ionic changes take place in the formed tissues in response to changes in the environmental fluid. However, it should be emphasized that *Pachygrapsus,* as an aquatic animal, must contend normally not only with salts and water reaching it by way of the gut but also with the flux of salts and water which occur continuously through permeable membranes separating the body fluids and tissues from the external medium, a problem not presented to terrestrial animals. It is interesting that in *Pachygrapsus* more than twice as much potassium is estimated to exchange between external medium and the crab, than the net exchanges calculated to occur in the "A–D volume" or probably the blood, itself (Fig. 1). Yet comparing the final blood concentrations with normal blood concentrations (Table I), the blood potassium does not vary from normal more than 30%. Thus, it seems that there is a tendency to maintain the blood potassium at a constant level at the expense of the tissue or pool potassium. This suggests a method of ionic regulation in the blood without need of a special organ such as a kidney.

It is possible, then, that the above described salt pools could have a special, functional significance which would be adaptive for an aquatic animal such as a crab. Thus, normally, osmotic and ionic constancy of the blood could be maintained at least partially by salt pools which are capable of mobilizing or fixing salts to and from the blood as the situation demands. Such a device would be necessarily of temporary value only, but would be particularly advantageous for estuarine forms which could make up an osmotic deficit from their salt pools at low tide and low salinities, then replenish the pools with a minimum of work when the salinity was elevated on the in-coming tide.

These studies were aided by a contract between the Office of Naval Research, Department of the Navy and the University of California, NR 163-309.

I am pleased to acknowledge the technical assistance of Mr. Paul Holland. Also I wish to express my gratitude to Professor Theodore Holmes Bullock for his advisory assistance in the preparation of the manuscript, to Professor Timothy Prout for his advice concerning the statistical handling of the data and to Professor Ralph Smith for his critical reading of parts of the manuscript.

Summary

1. When *Pachygrapsus* is immersed in a stress medium its blood concentration is altered by a loss of ions to a hypotonic medium and a gain of ions from a hypertonic medium. Water exchanges are insignificant in magnitude.

2. The observed ratio, change in blood ions/change in medium ions yields values for "apparent volume of distribution" for the respective ions. Such values vary according to the treatment for sodium and in moderate stresses average 38.5% body weight, in extreme stresses 45.9% body weight. For potassium most values came to more than 100% body weight and do not vary with increased stress. The above ratios are the same for a hypotonic medium as for a hypertonic medium.

3. Varying values for "apparent volume of distribution" under different magnitudes of osmotic stress suggest the presence of salt pools which may represent incidental participation of the formed tissues, or may represent an adaptive mechanism which functions to assist in the ionic and osmotic regulatory mechanism.

4. In stress media blood potassium and sodium are regulated equally well, percentage-wise, but a source other than the blood participates in the exchanges of potassium between animal and medium. Thus the potassium change in the blood does not account for the total potassium change in the animal.

5. "Apparent volume of distribution" calculated from the increased blood concentration caused by a given water loss by evaporation averages 48.9% body weight for sodium and only 13% body weight for potassium. The blood potassium therefore, increases percentage-wise about four times more than blood sodium. This indicates that potassium leaves a pool (probably the intra-cellular space) to enter the blood. This appears to be a physiological failure rather than regulation, and may play a role in ecological limitations.

6. Potassium concentrations in the blood of normal crabs (*Pachygrapsus*) are less than those of sea water. When immersed in dilute sea water of lower potassium concentrations than found in the blood of normal animals, the crabs usually tend to lose potassium so that it remains less concentrated than the potassium of the medium.

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